

A Single Dose of Methamphetamine Leads to a Long Term Reversal of the Blunted Dopamine D₁ Receptor-mediated Neocortical *c-fos* Responses in Mice Deficient for D₂ and D₃ Receptors*

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Dopamine D₁ receptors play an essential role in the induction of expression of the immediate-early gene *c-fos* in response to pharmacological stimuli. In the forebrain of wild-type mice, administration of a D₁ receptor agonist leads to *c-fos* mRNA expression levels that are substantially higher than corresponding levels expressed after indirect stimulation of dopamine receptors with methamphetamine. In mice deficient for D₂ and D₃ receptors, *c-fos* mRNA levels expressed in response to D₁ agonist administration are significantly blunted. However, a single dose of methamphetamine (5 mg/kg) leads to a long lasting reversal of the blunted *c-fos* responses in these mutants. In the forebrain, this reversal is restricted to the neocortex. Moreover, methamphetamine also enhances *c-fos* expression levels in preadolescent wild-type mice that normally express low *c-fos* mRNA in response to D₁ agonist stimulation. Thus, a single dose of methamphetamine leads to a long term increase in D₁ receptor-dependent *c-fos* responses in brains with either low (preadolescent mice) or blunted (adult D₂ and D₃ mutant mice) *c-fos* expression levels. A similar long term reversal of the blunted *c-fos* responses is achieved with a single dose of a full D₁ agonist. These results indicate that the constitutive inactivation of D₂ and D₃ receptors leads to a decrease in agonist-promoted D₁ receptor activity that can be reversed by intermittent agonist stimulation.

control of immediate-early gene expression by psychomotor stimulants, such as cocaine and amphetamine (3). Although D₁ receptors are essential for the induction of *c-fos* expression in response to psychostimulants, the magnitude of the D₁-dependent *c-fos* expression levels appears to be modulated by both D₂ and D₃ receptors. For example, the study of Moratalla *et al.* (3) identified anatomically restricted alterations in *c-fos* responses to haloperidol, a neuroleptic drug that blocks the D₂-like dopamine receptor subtypes D₂ and D₃, and other studies on mice deficient for D₃ receptors revealed blunted *c-fos* responses to D₁ agonist stimulation. These responses were even further reduced when D₃ mutants were pretreated with the D₂-like antagonist eticlopride (4).

To further investigate the role of D₂ and D₃ receptors in the modulation of *c-fos* responses to pharmacological stimuli, the present study used mice deficient for D₂ and D₃ receptors to analyze their levels of forebrain *c-fos* mRNA expressed in response to (a) direct stimulation of D₁ receptors with a full D₁ agonist, (b) indirect stimulation of dopamine receptors via methamphetamine-induced dopamine release, and (c) agonist stimulation of D₁ receptors 1–3 weeks after an application of either methamphetamine or the D₁ agonist. This study revealed that the constitutive inactivation of D₂ and D₃ receptors leads to a decrease in agonist-promoted D₁ receptor activity that can be reversed in a long term manner by a single dose of either methamphetamine or a D₁ agonist.

MATERIALS AND METHODS

Animals—The generation of D₂ and D₃ receptor knockout mice was described previously (5). The present study used the fifth generation of congenic C57Bl/6 mutants and their wild-type littermates. Experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Columbia University. All mice were housed in groups of 4–5 animals/cage with free access to food and water. Animals housed in the same cage received the same drug treatment (see below) and were returned to their home cage after drug injection until they were killed by decapitation.

Drug Treatments—All drugs were dissolved in saline and administered intraperitoneally. The D₁ agonist SKF82958, the D₁ antagonist SCH23390, and *S*(+)-methamphetamine hydrochloride were purchased from Research Biochemicals, Inc. (Natick, MA). The doses of methamphetamine (2–8 mg/kg) administered to the animals were calculated based on the molecular weight of the salt compound (C₁₀H₁₅N·HCl).

RNA Extraction and Northern Blotting—After decapitation, the brain was rapidly removed, and the forebrain was dissected. For this dissection, the mesodiencephalic junction was used as the anatomic landmark for the caudal border of the forebrain. In some experiments, the forebrain neocortex was further dissected from the extraneocortical structures containing the striatum, hypothalamus, thalamus, and epithalamus. RNA was extracted using the guanidine/cesium chloride ultracentrifugation method. 20 µg of total RNA (extracted from tissues pooled from 2 to 4 animals/genotype) was loaded onto each lane of

The induction of expression of the immediate-early gene *c-fos*, a gene with low base-line levels of expression in brain, is a well established and powerful tool for examining neuronal circuits that are activated biochemically in response to a variety of different stimuli. For example, studies on the induction of *c-fos* in response to an acute administration of drugs of abuse identified a common neuroanatomical pattern of expression (for review see Ref. 1), and it has been shown that the subchronic administration of such drugs (which is associated with a progressive sensitization of neuronal systems) leads to distinct alterations in the anatomic pattern of *c-fos* expression (2). In addition, studies on mutant mice have demonstrated that the expression of dopamine D₁ receptors is essential for the

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formaldehyde/agarose gels. Northern blots of these gels were probed with a ^{32}P -labeled random-primed cDNA corresponding to nucleotides 2160–2690 of the mouse *c-fos* gene (6). To confirm equal gel loading and membrane transfer of RNA, all blots were reprobed with a ^{32}P -labeled cDNA encoding the entire coding region of the human small nuclear ribonucleoprotein N gene that is expressed exclusively and at high levels in neurons (7). ^{32}P signals on autoradiograms were assessed densitometrically using the NIH Image Analysis software. Optical density measurements of standards on the film were made to construct a standard calibration curve. Relative optical densities were determined for optical densities of signals located in equal size sample areas, and the ratio of *c-fos*/N mRNA was determined for the signals on each lane. For animals at postnatal age 60 (P60)¹, the mean differences of mRNA levels between genotypes were determined with 4–5 independent Northern blotting experiments (each performed with RNAs pooled from two animals/genotype). For each series of these experiments, Northern blots were probed with equal aliquots of the same ^{32}P -radiolabeled *c-fos* cDNA and exposed to the same film. Multiple means of optical densities were compared with a one-way analysis of variance (ANOVA), and the significance of differences was assessed by Duncan's Studentized Range Test for comparisons of multiple means (threshold of significance, $p < 0.05$).

RESULTS

A first series of experiments determined the basal, D₁ agonist, and methamphetamine-induced *c-fos* mRNA levels in the forebrains of D₂ and D₃ mutant mice and their wild-type littermates. Compared with wild-type mice (in which *c-fos* mRNA levels are undetectable), the basal *c-fos* mRNA levels are higher in the forebrains of both D₂ and D₃ mutant mice (Fig. 1A).

The induction of *c-fos* mRNA expression was determined 60 min after application of the full D₁ agonist SKF82958 (1 mg/kg). This induction is robust in wild type, but by comparison it is drastically blunted in both D₂ and D₃ mutants (Fig. 1A). A comparison of optical densities (OD) determined for equal size fields of the autoradiogram shown in Fig. 1A (OD wild type, 6.0; D₂ mutants, 0.5; D₃ mutants, 0.8) indicates that the *c-fos* responses of D₂ and D₃ mutants are reduced to 8.3 and 13.3%, respectively, of the corresponding wild-type level. In five independent experiments (see "Materials and Methods"), *c-fos* mRNA levels expressed in D₂ mutants were only $12.2 \pm 3.7\%$ of the corresponding wild-type levels ($p < 0.001$), and *c-fos* mRNA levels of D₃ mutants reached only $15.7 \pm 9.4\%$ of levels expressed in wild type ($p < 0.001$). *c-fos* mRNA levels expressed in D₂ and D₃ mutants did not differ significantly (Table I).

Another series of experiments measured *c-fos* mRNA levels expressed in response to a single dose of methamphetamine (8 mg/kg). In the forebrain of wild-type mice, *c-fos* responses are substantially lower than corresponding responses to the D₁ agonist (Fig. 1A). A comparison of optical densities on the autoradiogram shown in Fig. 1A indicates that the magnitude of these *c-fos* responses (OD, 1.0) is only 16.7% of the responses detected in wild-type animals after D₁ agonist application (OD, 6.0). In four independent experiments, *c-fos* levels expressed in methamphetamine-treated wild-type mice reached only $23.7 \pm 12.0\%$ of the corresponding levels expressed in SKF-treated wild-type mice (Student's *t* test, $p < 0.001$). *c-fos* mRNA levels induced with only 2 mg/kg methamphetamine also do not differ from *c-fos* mRNA levels induced with 8 mg/kg methamphetamine (data not shown). Moreover, in contrast to the results obtained with the D₁ agonist, methamphetamine-induced *c-fos* responses of D₂ or D₃ mutant mice do not differ from wild type (Fig. 1A). Nevertheless, as shown in Fig. 1B in wild type and D₂ and D₃ mutants, *c-fos* responses to methamphetamine treatment are markedly reduced by pretreatment with the D₁ receptor antagonist SCH23390 (0.3 mg/kg), but they are unaf-

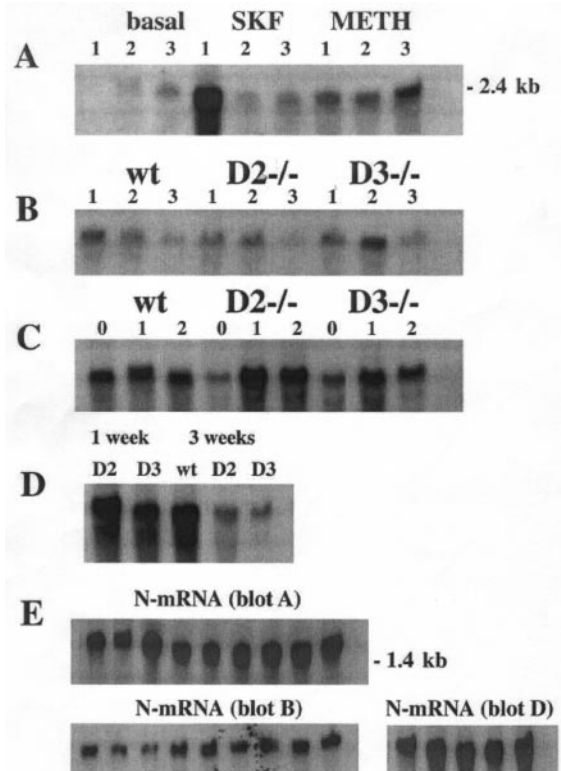


FIG. 1. Basal, D₁ agonist, and methamphetamine-induced *c-fos* mRNA responses in the forebrains of adult (P60) wild-type mice and mice deficient for D₂ and D₃ receptors. A, basal *c-fos* mRNA levels of wild type (lane 1), homozygous D₂ (lane 2), and D₃ mutants (lane 3) were determined 60 min after an intraperitoneal saline injection. *c-fos* responses to an intraperitoneal injection of the D₁ agonist SKF82958 (1 mg/kg) and methamphetamine (8 mg/kg) in wild type (lanes 1), D₂ (lanes 2), and D₃ mutants (lanes 3) were also measured 60 min after drug administration. A 0.24–9.5-kilobase RNA ladder (Life Technologies, Inc.) indicates a ~2.3-kilobase *c-fos* mRNA species that is known to encode the *c-fos* protein. B, *c-fos* responses to methamphetamine of wild type (wt), homozygous D₂ (D₂^{-/-}), and D₃ (D₃^{-/-}) mutants (lanes 1) are unaffected by pretreatment with saline (lanes 2), but they are markedly reduced by pretreatment with the D₁-selective antagonist SCH23390 (0.3 mg/kg intraperitoneal) (lanes 3). C, *c-fos* responses to SKF (1 mg/kg) administered 1 and 2 weeks after a single injection of methamphetamine (5 mg/kg intraperitoneal) (lanes 1 and 2, respectively) in wild type, homozygous D₂, and D₃ mutants compared with corresponding responses measured in drug-naïve animals (lanes 0). D, when SKF is administered 3 weeks after methamphetamine pretreatment, blunted *c-fos* responses of D₂ and D₃ mutants are again apparent when compared with wild type. E, to test for possible differences in the amounts of mRNAs loaded onto and/or transferred from each lane of the gels, all blots were reprobed with c-DNA encoding the brain-specific small nuclear ribonucleoprotein N (7). Representative examples shown for; blots A, B, and D illustrate that equal levels of the 1.6-kilobase N-encoded mRNA are detected on all lanes.

ected by a pretreatment with saline. These data confirm that the induction of *c-fos* expression by amphetamine-like drugs is dependent upon D₁ receptor activation (3).

Additional studies compared *c-fos* responses to D₁ agonist stimulation in drug-naïve mice and mice that received a single dose of methamphetamine (5 mg/kg) 1, 2, or 3 weeks before SKF treatment. In wild-type animals, the *c-fos* responses to D₁ agonist stimulation are not significantly altered 1 and 2 weeks after methamphetamine pretreatment (Fig. 1C). Interestingly however, as many as 2 weeks after a single dose of methamphetamine, the levels of *c-fos* induced in both D₂ and D₃ mutants are similar to the levels expressed in either drug-naïve or methamphetamine-pretreated wild-type mice (Fig. 1C). Thus, in contrast to the blunted *c-fos* responses to D₁ agonist stimulation of drug-naïve mutants (Fig. 1A), methamphetamine-

¹ The abbreviations used are: Pn, postnatal day n; OD, optical density; MAP, mitogen-activated protein.

TABLE I

Comparison of ODs of *c-fos* mRNA signals of SKF82958-treated drug-naive, methamphetamine (METH)- and SKF-pretreated wild type, and D_2 and D_3 mutant mice

Data were obtained from SKF-treated drug-naive animals (SKF) and from methamphetamine- or SKF-pretreated animals that received a challenge dose of SKF (METH/SKF and SKF/SKF, respectively).

Treatment	Brain region	Wild type ODs	D_2 -/- ODs	D_3 -/- ODs
SKF	Forebrain	5 ± 1.5	0.6 ± 0.2 ^a	0.7 ± 0.2 ^a
METH/SKF	Neocortex	2.1 ± 0.5	3.9 ± 1.7 ^b	3.9 ± 1.7 ^c
SKF/SKF	Neocortex	0.9 ± 0.1	1.8 ± 0.3 ^a	1.3 ± 0.1 ^{b,d}

^a $p < 0.001$ compared with wild type.

^b $p < 0.01$ compared to wild type.

^c $p < 0.05$ compared with wild type.

^d $p < 0.01$ compared with D_2 mutants.

pretreated D_2 and D_3 mutants show substantially more robust *c-fos* responses to D_1 agonists (see below). However, 3 weeks after methamphetamine administration, the blunted *c-fos* responses of the mutants to D_1 agonist stimulation are again apparent, and a comparison of the optical densities of signals on the autoradiogram shown in Fig. 1D (OD wild type, 4.08; D_2 mutants, 0.8; D_3 mutants, 1.0) indicates that D_2 and D_3 mutants express only 19.5 and 24.4% of the corresponding wild-type *c-fos* levels (these levels are similar to the levels determined above for SKF-treated drug-naive mutants).

The results summarized above were obtained from adult mice that received the first drug injection at P60. To determine whether similar results were obtained in preadolescent mice, experiments were also performed in mice at postnatal age 30. As shown in Fig. 2A, P30 mice express only marginally increased levels of *c-fos* in response to the D_1 agonist (1 mg/kg), and these levels do not differ from corresponding levels induced by methamphetamine (8 mg/kg). Moreover, at these low expression levels, no significant differences are found between wild type and D_2 mutants, and D_3 mutants express only slightly reduced levels of *c-fos* in response to both SKF and methamphetamine (Fig. 2A, see lanes marked 1, 2, and 3). However, when P30 mice (both wild type and mutants) are treated with a single dose of methamphetamine (5 mg/kg) and challenged with SKF at P47, *c-fos* responses are robust in all genotypes, and no differences are found between wild-type and mutant mice. Furthermore, D_2 single mutants that received methamphetamine at postnatal day 24 also express high levels of *c-fos* in response to SKF administered at P30, and the *c-fos* mRNA levels of these mutants do not differ from the levels expressed in mice treated with methamphetamine at P30 and challenged with SKF at P47 (Fig. 2B). Altogether, these results indicate that a single dose of methamphetamine leads to a long term increase in *c-fos* responses to D_1 agonist stimulation in brains with either low (preadolescent mice) or blunted (adult D_2 and D_3 mutant mice) *c-fos* expression levels.

To further determine whether the D_1 agonist and methamphetamine induce different levels of *c-fos* in different anatomic areas of the forebrain, additional experiments compared *c-fos* mRNA levels in the neocortex and remaining forebrain of adult wild-type and mutant mice. These results revealed that the levels of *c-fos* mRNA expressed in response to a single dose of methamphetamine are higher in the forebrain neocortex compared with the inner (extraneocortical) mass of the forebrain (Fig. 3). This finding is in contrast to the D_1 agonist, which induces similar levels of *c-fos* in the neocortex and in extraneocortical forebrain structures (Fig. 4). Moreover, in both D_2 and D_3 mutants that were pretreated with methamphetamine, drastically enhanced *c-fos* responses to SKF treatment are detected in the neocortex, but the *c-fos* mRNA levels in the extraneocortical forebrain remain low (Fig. 3). In fact, on the autoradiogram shown in Fig. 3, neocortical *c-fos* levels of D_2

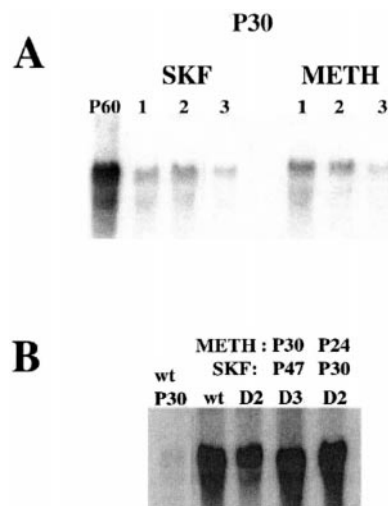


FIG. 2. *c-fos* mRNA responses to a D_1 agonist and methamphetamine in the forebrains of wild-type, D_2 , and D_3 mutant mice at postnatal age P30. A, the *c-fos* mRNA levels expressed in P30 wild type (lanes 1), homozygous D_2 mutants (lanes 2), and homozygous D_3 mutants (lanes 3) in response to SKF82958 (SKF, 1 mg/kg) and methamphetamine (METH, 8 mg/kg) are compared with *c-fos* mRNA levels expressed in SKF-treated D_3 mutants at P60 (lane P60). B, *c-fos* mRNA levels of SKF-treated P30 wild-type mice (wt P30) are compared with corresponding mRNA levels expressed in wild-type (wt), homozygous D_2 , and homozygous D_3 mutant mice that received a single injection of methamphetamine (5 mg/kg) at P30 followed by a single dose of SKF (1 mg/kg) at P47, and D_2 mutants that received an equal dose of methamphetamine at P15 followed by SKF treatment at P30. For each genotype, brain tissues of four animals/genotype were pooled for RNA extraction, and 20 μ g of total RNA was loaded onto each lane. The blots shown in A and B were exposed to film for 16 and 6 h, respectively.

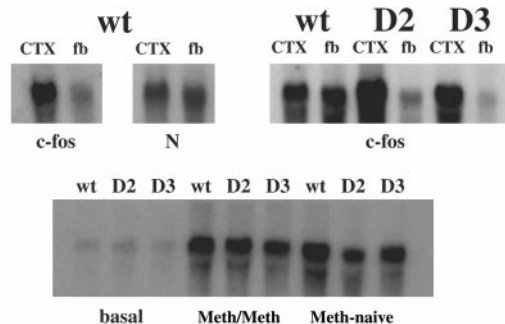


FIG. 3. *c-fos* mRNA responses to methamphetamine and SKF82958 in the neocortex and extraneocortical forebrain structures of wild-type, D_2 , and D_3 mutant mice. Top left, *c-fos* mRNA levels detected in the neocortex (CTX) and extraneocortical forebrain (fb) 60 min after methamphetamine (8 mg/kg) treatment of P60 wild-type (wt) mice. Top middle, the blot shown on the left was reprobbed with radiolabeled N-encoded cDNA. Top right, comparison of *c-fos* mRNA levels expressed in response to SKF treatment in the neocortex and in extraneocortical forebrains of wild type, homozygous D_2 (D_2), and D_3 (D_3) mutants that were treated with a single dose of methamphetamine (5 mg/kg) 1 week before SKF administration. Bottom, the first three lanes show basal levels of *c-fos* mRNA detected 1 week after the administration of a single dose of methamphetamine. The following lanes show *c-fos* responses to methamphetamine (8 mg/kg) given to either methamphetamine-pretreated animals (Meth/Meth) or drug-naive animals (Meth-naive). 20 μ g of total RNA was loaded onto each lane, and all blots were exposed to film for 6 h.

and D_3 mutants are 3.4- and 2.1-fold higher, respectively, compared with wild-type *c-fos* levels (OD wild type, 1.37; D_2 mutants, 4.70; D_3 mutants, 2.87). By comparison, the optical densities of *c-fos* signals obtained from extraneocortical forebrain mRNA are 1.56, 0.5, and 0.5 for wild type, D_2 mutants, and D_3 mutants, respectively. Thus, the methamphetamine-induced reversal of the blunted *c-fos* expression of both mutants in

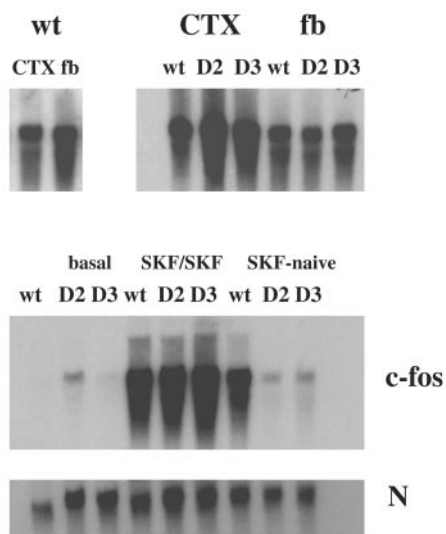


FIG. 4. *c-fos* mRNA responses to SKF82958 in the neocortex and extraneocortical forebrain structures of SKF-naive and SKF-pretreated wild-type, D₂, and D₃ mutant mice. *Top left*, *c-fos* mRNA levels expressed 60 min after SKF injection in the neocortex (CTX) and in the extraneocortical forebrain (fb) of SKF-naive wild types (wt). *Top right*, *c-fos* mRNA expressed in wild-type and mutant mice that were treated with a single dose of SKF (1 mg/kg) 1 week before a subsequent dose of SKF. *Middle*, the first three lanes show basal levels of *c-fos* mRNA detected 1 week after the administration of a single dose of SKF. The following lanes show *c-fos* responses to SKF given either to SKF-pretreated animals (SKF/SKF) or to SKF-naive animals (SKF-naive). *Bottom*, the blot shown in the middle was reprobbed with N-encodded cDNA. 20 μ g of total RNA was loaded onto each lane, and all blots were exposed to film for 3 h.

response to D₁ agonist treatment is only due to an increase in their neocortical *c-fos* mRNA responses. In four independent experiments (see Table I), neocortical *c-fos* mRNA levels of D₂ mutants were $310 \pm 40\%$ of the corresponding wild-type levels, and this difference is significant ($p < 0.01$). Mean neocortical *c-fos* mRNA levels of D₃ mutants were also significantly increased ($229 \pm 39.4\%$ of corresponding wild-type levels, $p < 0.05$).

Pretreatment with methamphetamine, however, does not affect *c-fos* responses to subsequent stimulation with methamphetamine. As shown in Fig. 3, methamphetamine-induced *c-fos* responses are indistinguishable between drug-naive and methamphetamine-pretreated wild-type and mutant mice. Interestingly however, as also shown in Fig. 3, the basal levels of *c-fos* mRNA measured 1 week after methamphetamine administration no longer differ between wild-type and mutant mice (compare with Fig. 1A).

Another experiment tested whether pretreatment with a single dose of the D₁ agonist (1 mg/kg) also affects *c-fos* responses to subsequent D₁ agonist stimulation. These results are shown in Fig. 4. In both neocortex and extraneocortical forebrains, *c-fos* induction by SKF treatment is robust in wild type and in D₂ and D₃ mutants that were pretreated with SKF 1 week earlier. In fact, compared with wild type, optical densities of the signals on the autoradiogram shown in Fig. 4 (*top right*) revealed a 1.8- and 1.3-fold increase in neocortical *c-fos* mRNA levels expressed in D₂ and D₃ mutants, respectively. In four independent experiments (see Table I), neocortical *c-fos* mRNA levels of D₂ and D₃ mutants were significantly increased to $201 \pm 10.2\%$ and $151 \pm 30.6\%$, respectively, of the corresponding wild-type levels (D₂ mutants/wild type, $p < 0.001$; D₃ mutants/wild type, $p < 0.01$; D₂ mutants/D₃ mutants, $p < 0.01$).

A further comparison between *c-fos* responses of SKF-naive and SKF-pretreated animals revealed that SKF pretreatment, similar to methamphetamine pretreatment, markedly increased *c-fos* responses only in the mutants (Fig. 4, *bottom*). However, in contrast to methamphetamine, SKF does not significantly alter the increased basal levels of *c-fos* in D₂ mutants. Also, D₃ mutants continue to express detectable *c-fos* mRNA, although to a lesser extent (Fig. 4, *bottom*).

DISCUSSION

This study shows that compared with methamphetamine, higher *c-fos* mRNA levels are expressed in response to D₁ agonist stimulation and that adult mice lacking either the D₂ or D₃ receptor show blunted *c-fos* responses to the D₁ agonist. A single dose of methamphetamine induces a long lasting enhancement of *c-fos* responses in brains with either low (preadolescent wild-type and mutant mice) or blunted (adult D₂ and D₃ mutants) *c-fos* expression levels. Moreover, the enhanced *c-fos* responses to the D₁ agonist seen in methamphetamine-pretreated adult mutants are only detected in the neocortex, a brain region in which the acute administration of methamphetamine itself induces the largest *c-fos* responses. Furthermore, a single dose of a full D₁ agonist elicits similar long lasting enhancement of *c-fos* responses to subsequent D₁ agonist stimulation. These data suggest that despite the unaltered expression of D₁ ligand-binding sites in D₂ and D₃ mutants (8, 9), the chronic inactivation of D₂ and D₃ receptors leads to a decreased responsiveness of D₁ receptors to agonist stimulation, which can be reversed by a single dose of either methamphetamine or a D₁ agonist.

Significantly reduced *c-fos* protein responses to D₁-agonist stimulation were previously reported (4) for the same D₃ mutants in this study. The present analysis of *c-fos* mRNA expression levels revealed similarly blunted *c-fos* responses in mice deficient for D₂ receptors, an effect that could only marginally be detected in the previous protein study (4). This analysis suggests that D₂ but not D₃ mutants develop compensatory mechanisms that operate either at the translational or post-translational level to maintain wild-type-like *c-fos* protein responses to D₁ agonist stimulation. The present study also found increased basal levels of *c-fos* mRNA in the forebrain of mice deficient for D₂ and D₃ receptors. This result is similar to the results of previous pharmacological studies showing increased *c-fos* expression levels in rats that were treated acutely or chronically with the D₂/D₃ receptor blocker haloperidol (10, 11).

The *c-fos* responses to methamphetamine differ both quantitatively and qualitatively from the *c-fos* responses induced by the D₁ agonist. In contrast to the widespread *c-fos* expression induced by the D₁ agonist, the effects of methamphetamine are delimited to the neocortex. These effects suggest that the two types of pharmacological stimuli activate different neuronal populations/circuitries that express *c-fos*, and future and more detailed investigations of the anatomic distribution of *c-fos* mRNA expression will need to test investigation. The quantitative differences of *c-fos* expression levels induced by methamphetamine and D₁ agonists may also reflect differences in the activation of the two principal transcriptional activators of the FOS gene, pMAP kinase and cAMP-response element-binding protein (pCREB) (12). In any case, a main finding of the present study is that a single dose of methamphetamine (5 mg/kg) leads to a long term (as many as 2 weeks) reversal of the blunted *c-fos* responses to D₁ agonist stimulation in the forebrain of mice deficient for D₂ and D₃ receptors. Interestingly, the same dose of amphetamine has previously also been shown to induce a long-lasting behavioral and neuroendocrine sensitization in rats that is accompanied by an increase in electrically evoked dopamine release in the forebrain (13). Moreover,

as shown here one long term consequence of this dose of methamphetamine is a decrease of the (abnormally) high basal *c-fos* levels in D₂ and D₃ mutants. This consequence may perhaps be one of the mechanisms by which methamphetamine (but not the D₁ agonist) increases the responsiveness of neurons to subsequent D₁ agonist stimulation.

It will be of great interest to further elucidate the molecular mechanisms that lead to the methamphetamine-induced and D₁ agonist-induced long term enhancement of cortical *c-fos* responses to D₁ agonist stimulation in brains with low or abnormally blunted *c-fos* responses. The decreased agonist-promoted D₁ receptor activity detected in D₂ and D₃ mutants suggest that the chronic treatment with neuroleptic drugs that block D₂ and D₃ receptors (a common therapeutic intervention of schizophrenia) could impair the function of D₁ receptors. Several other studies provided evidence for a reduced cortical D₁ receptor activity during chronic neuroleptic treatment (14–16), and results of a most recent study (14) suggested for the first time that a short term co-administration of a D₁-selective agonist to monkeys chronically treated with neuroleptics can improve behavioral deficits associated with a decreased cortical D₁ receptor activity. The present study now shows that the decreased response of neocortical D₁ receptors to agonist stimulation in mice deficient for D₂ and D₃ receptors is not irreversible and suggests that an intermittent stimulation of dopamine release by amphetamine-like drugs during treatment with typical neuroleptics can result in a long term increase in agonist-promoted D₁ receptor activity.

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